



# Non-Targeted Analysis (NTA) : What and Why - PFAS as a case in point

**Mark Strynar**

U.S. EPA National Exposure Research Lab  
Exposure Methods & Measurements Division

Briefing for David Dunlap  
November 6<sup>th</sup>, 2018



U.S. Environmental Protection Agency

## Outline

- Context/Motivation
- Description of NTA
- NTA as applied to PFAS – EPA Examples
- Summary

# NTA Measurement Context

- Targeted Analysis:

- We know exactly what we're looking; have a chemical standard
- 10s – 100s of chemicals



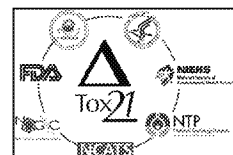
- Suspect Screening (SSA):

- We have chemical lists
- 100s – 1,000s of chemicals

- Non-Targeted Analysis (NTA):

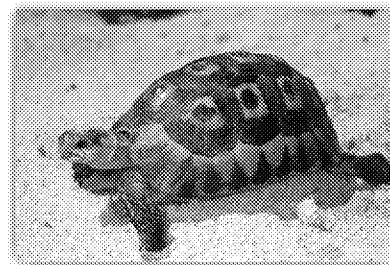
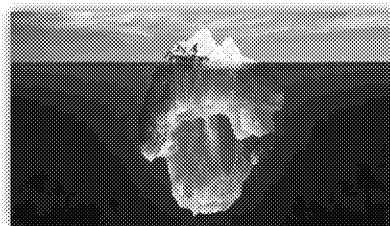
- We have no chemical lists
- 1,000s – 1,000,000s of chemicals
- In dust, soil, food, products, plants, animals

High  
Resolution  
Mass  
Spectrometry



## Motivation for NTA

- Targeted analysis represents small fraction of anthropogenic chemical exposure, i.e.  $<<1\%$
- Provides means to identify exposure to chemicals of emerging concern
- Time to acquire targeted analysis lags release, occurrence, exposure, and risk by years
  - Need a standard, method development, validation
- We can't protect human health/environment from 21st century threats if we're blinded to +99% of exposures and lag exposure by years



## Is Nontargeted Screening Reproducible?

Ronald A. Hites<sup>\*✉</sup>

School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana 47405, United States

Karl J. Jobst<sup>\*</sup>

Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario L8S 4M1, Canada

“(d) The last step is the most important: One should answer the classic “So what?” question. What is this compound doing in that sample? How did it get there? Is this compound widely produced? How widespread is it in the environment, or is its presence site specific? Answering these questions usually requires developing a quantitative method using authentic standards.

## ORD Leadership in the Scientific Community: EPA's Non-Targeted Analysis Collaborative Trial (ENTACT)

### Questions to be Addressed:

- How variable are tools and results from lab to lab?
- Are some methods/tools better than others?
- Does sample complexity affect performance?
- What chemical space does a given method cover?
- Can we comprehensively characterize any sample?
- How does chemical content vary across media?

### Collaborators



### Example Activities:

- Organized and Hosted 2 international NTA workshops in RTP, NC (2015, 2018)
- Multi-lab Round Robin: Provided to collaborators for SSA/NTA investigations
  - Chemical mixtures (n=10)
  - Standard reference materials
  - SRMs (house dust/human serum) from NIST (spiked/unspiked)
  - DSS-TOX Chemicals Database

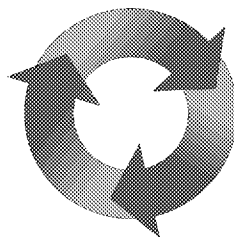
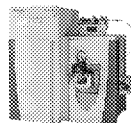
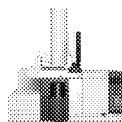
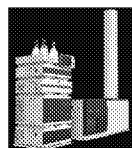
## NTA Relevance/Responsiveness to Agency Problems

- Agency Programs
  - OCSP-P-TSCA
  - OLEM-CERLCA
  - OW-Safe Drinking Water Act
  - OAR-Clean Air Act
  - Cooperative Federalism
- ORD CSS Program
  - Provide rapid and efficient approaches to prioritize, screen, and evaluate chemicals for safety using scientifically-sound, transparent processes.

# NTA Essential Components

## Analytical Instruments

Orbitrap; QToF (recent equipment)



## Comp. Tools & Workflows

FOR  
IDENT

Xcms  
Online

Metrag

## Chemical Databases

ChemSpider  
The free chemical database

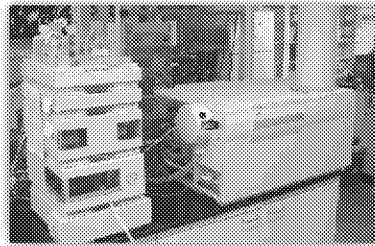
PubChem

MassBank



## Application to Broad Range of Media

- Water
  - industrial effluent, surface, source, well, drinking, Brita® filters
- Solids
  - Soil, sediment, house dust, char, food packaging
- Animal tissue (fish, mouse, bird)
- Air (gearing up)
  - Stack, ambient, & indoor air
- Human
  - Blood & urine



LC/TOFMS

# The Vast PFAS Unknown

Increasing Environmental, Regulatory and Public Health Attention

All PFAS (n = 3000 - 5000)

Common State Analytes\*

UCMR3

Per and polyfluorinated

Perfluorinated ("PFCs")

Substituted  
PFH<sub>x</sub>S\*, PFOS\*, PFDS\*

Carbonates  
PFNA\*, PFHpA\*

Early Attention  
PFOS\* PFOA\*

Recent Attention

Fluorotelomers  
PF<sub>n</sub>DA, PF<sub>n</sub>DB,  
PFDoA, PFUnA,  
PFGeA, PFTrDA,  
PFTrDA, PFEnA,  
PFGEA

Increasing Attention

Future Attention

All Other PFAS

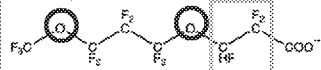
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50 F St., NW, Suite 550  
Washington, DC 20001  
frc@etrcc.org



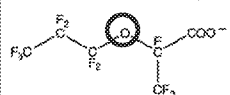
**ITRC**  
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Washington, DC 20001  
ibroweb.org

## Fluoropolymer manufacture

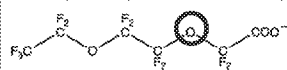
ADONA (CAS No. 958445-44-8)



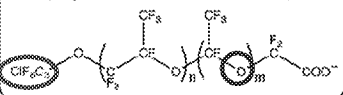
GenX (CAS No. 62037-80-3)



Asahi's product (CAS No. 908020-52-0)

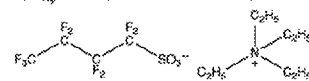


Solvay's product (CAS No. 329238-24-6)

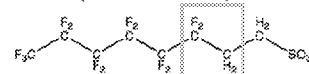


## Metal plating

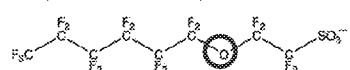
N(Et)<sub>4</sub>-PFBS (CAS No. 25628-06-4)



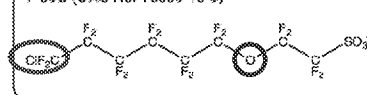
6:2 FTSA (CAS No. 27615-97-2)



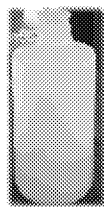
F-53 (CAS No. 754825-54-7)



F-53B (CAS No. 73606-19-6)

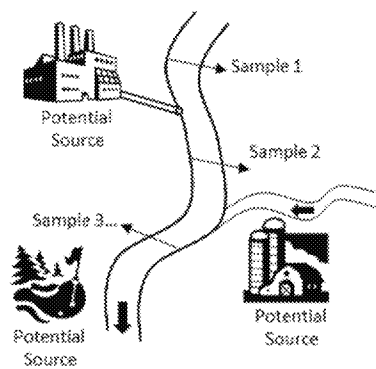


Surface Water  
Ground Water  
WWTP Effluent  
Drinking Water

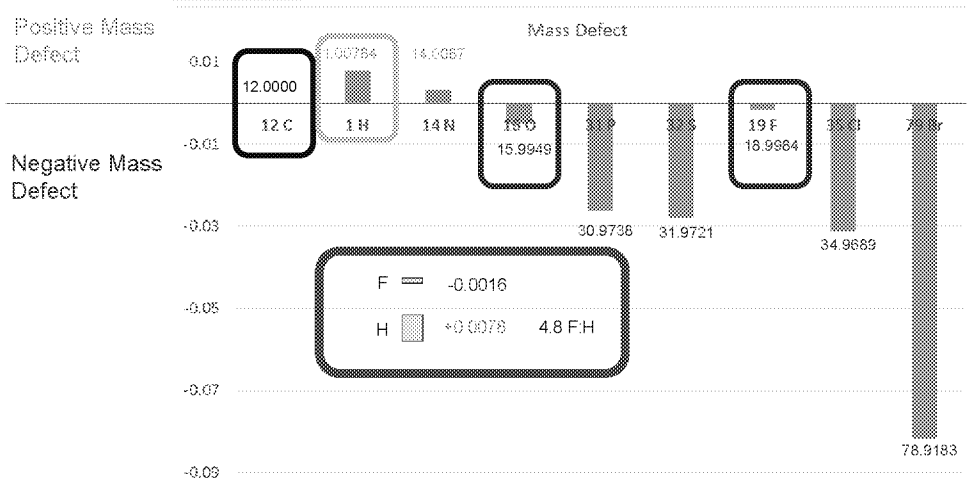


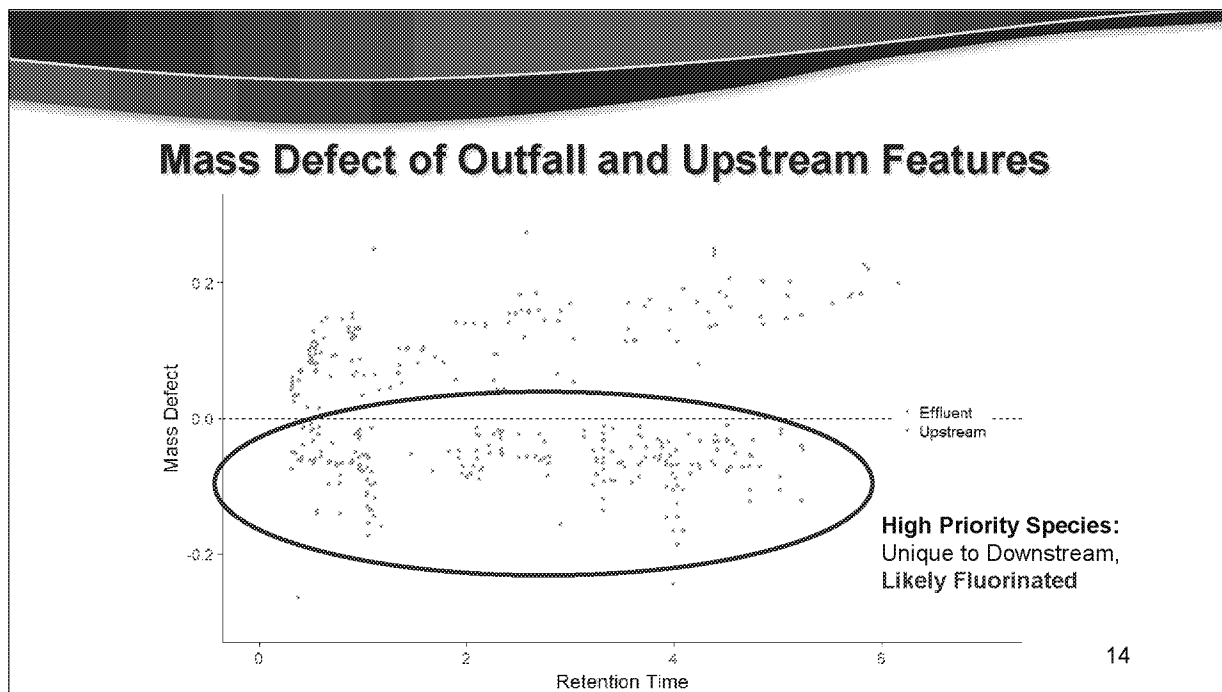
HDPE Bottles

Sampling from geographically or temporally displaced locations allows triangulation of sourcing



# Isotope Signatures: Negative Mass Defect

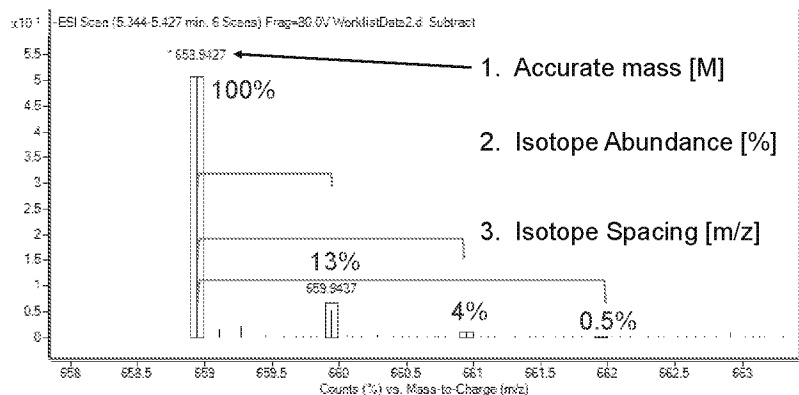
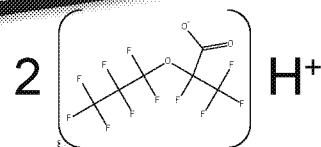




MAKE ONE OF THESE FOR OUR DATA

# Isotope Cluster Scoring

Ex.  $C_{12}H_7F_{22}O_2$



Strynar et al., 2015 ES&T

## PFAS in the NC Cape Fear River

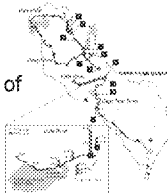




# Method of Analysis in Brief

## • Study Design

- Water sampling (discharge, surface, source, drinking) up- and down-stream of source
- Before and after industry actions to remediate discharge

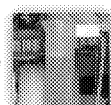


## • Extraction

- 1 L HDPE bottle with HNO<sub>3</sub> (35%): Shipped ambient

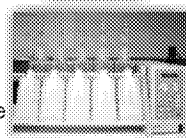


- Volume measured; sample bottle washed with MeOH



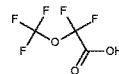
- Sample filtered

- Sample loaded onto SPE tube



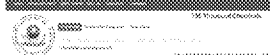
## • Non-Targeted Analysis

- High resolution mass spectrometry
- Mass assigned to each peak observed, e.g. 179.9846 Daltons (Da)
- Software calculates exact number and type of atoms needed to achieve measured mass, e.g. C<sub>3</sub>HF<sub>5</sub>O<sub>3</sub>
- Software and fragmentation inform most likely structure



Molecular Formula: C<sub>3</sub>HF<sub>5</sub>O<sub>3</sub>  
 Monoisotopic Mass: 179.9846 Da  
 (m/z)

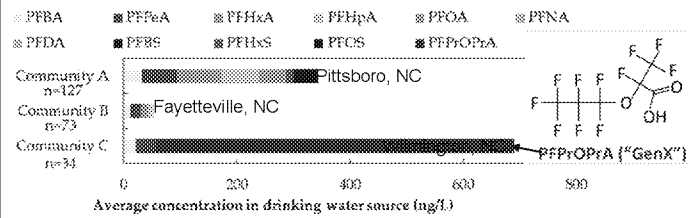
- With mass, formula, structure known, potential identities determined by database search



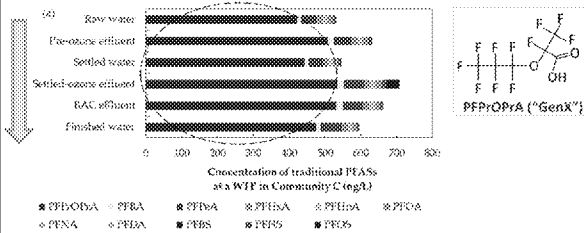
Source: Strynar et al. 2015; Sun et al. 2016

# PFAS Perspective Gained by NTA

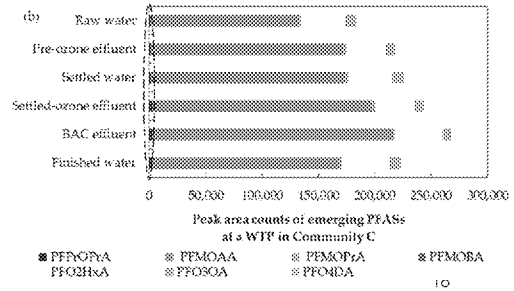
## • GenX found in drinking water downstream of Chemours



## • Not removed through conventional treatment



## • And "tip of the iceberg" of total PFAS present



Source: Strynar et al. 2015; Sun et al. 2016

## A Case-in-Point for NTA Work that was Relevant, Actionable, and Impactful

- Timeline of GenX Contamination Discovery and Response:**

  - (Nov 2016)** Science & Technology magazine article: "Legacy and Emerging Perfluorinated Substances Are Important Drinking Water Contaminants in the Cape Fear River Watershed of North Carolina"
  - GenX quantified in drinking water**
  - Local news media picks up research reports**
  - Chemours mitigates GenX discharge to Cape Fear River**
  - NC DEQ, EPA (including Region 4 and ORD) partner to monitor mitigation effectiveness**
  - Partnership continues: Tech transfer to R4 GenX analysis; air emissions monitoring**

**Supporting Information:**

  - NEW TEFLON TOXIN FOUND IN NORTH CAROLINA DRINKING WATER** (June 2017)
  - Chemours Says It Will Take Additional Steps to Keep GenX from Cape Fear River as EPA, State Begin Investigations** (June 2017)
  - GenX in Drinking Water** (Graph showing GenX concentration over time)
  - Nafion byproduct 1** and **Nafion byproduct 2** (Chemical structures)
  - GenX (C<sub>6</sub>H<sub>5</sub>F<sub>5</sub>O<sub>2</sub>S<sub>2</sub>F<sub>6</sub>)** (Chemical structure)

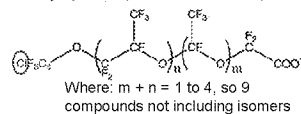
## Helping States Solve Problems

**Common theme / question:** Is there environmental contamination from air and water emissions of novel PFAS warranting further investigation?

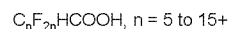
- NJ DEP / R2
  - Concern over emissions from Solvay and Chemours
- NH DES / R1
  - Concern over emissions from Saint-Gobain Performance Plastics (SGPPL, Merrimack) and the Textile Coating International (TCI, Manchester)
- WV/ R3
  - Concern over Chemours air emissions
- MI & MN/ R5
  - Concern over chrome plating industry

### NTA Preliminary Discoveries

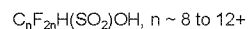
Solvay's product (CAS No. 329238-24-5)



H substituted polyfluorinated carboxylic acid series



H substituted polyfluorinated sulfonic acid series



## NTA Scientific and Communication Challenges

- Transparency as to limitations
  - Identification < 100% certain (lack of standards)
  - Concentration (e.g. 10X uncertainty)
- Lack of familiarity with what / how
- Results semi-quantitative
- Chemical identification is manual, highly specialized, time/effort intensive
- Public health interpretation given lack of toxicity information
- Role of NTA in advancing the Agency's mission

## Summary

- NTA is a powerful tool which compliments targeted analysis and is in high demand for evaluation of chemical exposure
  - Expansive chemical window on to exposure
  - Reflects actual contemporary exposure
  - Relevant to future Agency regulatory needs
- NTA is a specialized ORD capability under high demand by local, states, and regions, especially related to PFAS
- Communication strategy is key consideration – capabilities, limitations, and uncertainty
- NTA plays an important role in discovering and identifying emerging chemicals for investigation, risk assessment, and action where needed

22

## Who is Doing the Hard Work

- **Technical Experts**
  - Andy Lindstrom
  - Mark Strynar
  - Seth Newton
  - John Washington
  - James McCord
  - John Offenberg
  - Theran Riedel
  - Jeff Ryan
- **QA Managment**
  - Sania Tong-Argao
  - Margie Vazquez
  - Brittany Stuart
- **Communications**
  - Emily Smith
- **Coordination/Mgmt**
  - Becky Allenbach (R4)
  - Linda Culpepper (NC DEQ)
  - Myriam Medina-Vera
  - Adam Biales
  - Brian Schumacher
  - Surender Kaushik

# **Extra Slides if Needed**

24



# NTA Informs Exposure

NTA provides a powerful tool with current, expansive, and unbiased window on to chemical exposure to inform risk and epidemiology

## Too many chemicals

- The number of chemicals that we produce, emit to the environment, or use in consumer product formulations vastly exceeds our ability to assess exposure, hazard, or risk (Egeghy et al. 2011)

## Large health burden poorly understood

- "Chemical pollution is a great and growing global problem. The effects of chemical pollution on human health are poorly defined and its contribution to the global burden of disease is almost certainly underestimated." Landrigan et al., 2017

A. Breakdown of the Chemicals in commerce - USA



B. "Industrial" Chemicals in commerce - US TSCA inventory

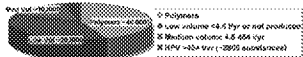
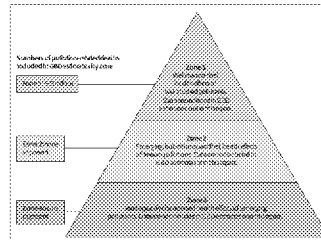


FIGURE 1. Individual number and categories of chemicals in commerce registered for use in the United States over the past 30 years. Not all chemicals are in the same year. Smaller proportions would be indicated in other countries.

Source: Muir and Howard, 2006



From: The pollution

Source: Landrigan et al, 2017



25

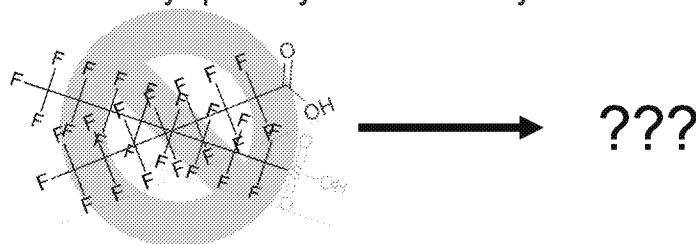
OCSPP estimates active inventory of TSCA chemicals to be 38,000

## Ongoing PFAS NTA Work

- MN / MI / R5
  - Concern over emissions from electroplating operations mist suppressants
  - In planning
- Ohio State Univ Collaboration
  - Chemours site in Parkersburg W VA
  - Water & soil sampling of GenX implicating air emissions
  - Manuscript in development
- NCSU / UNC Collaboration NIEHS R21 Grant
  - NTA of drinking water, urine, and blood from Wilmington residents
  - Methods being developed / applied

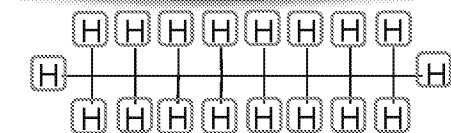
## OTHER PFASs: The Era of HRMS & Non-Targeted Analysis

- How do we find compounds without knowing what they are?
- How do we prioritize unknowns for further analysis?
- How do we identify/quantify without analytical standards?

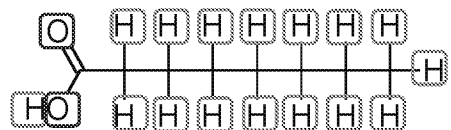


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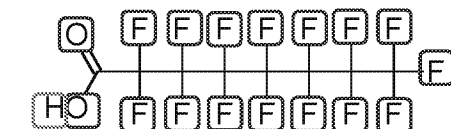
## Example of Mass Defect



Octane  
MI mass 114.1409



Octanoic Acid  
MI mass 144.1150



Perfluorooctanoic Acid  
MI mass 413.9737

**AFFF**

28

Chemistry Dashboard | Home

Secure | comptox

Apps | GreenCode | Chemistry Dashboard | NORMAN Support | FOR-IDENT - Home | Meeting | CFM-10 | Search Mass Calculator | Software - Explore | Open Portal

EPA United States Environmental Protection Agency

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Chemicals | Product/Use Categories | Assay/Gene

Search by chemical name, CAS number, or other identifiers

Identifier subsetting search

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**New publication released: "MS-Ready" structures for non-targeted high-resolution mass spectrometry screening studies**

August 21st, 2018 at 12:07:24 PM

"MS-Ready" structures are the basis of many of the searches to support mass spectrometry that are supported on the dashboard. Our recent publication "MS-Ready" structures for non-targeted high-resolution mass spectrometry screening studies" explains the concept and production of MS-Ready structures in detail. [Read this paper here](#)

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5:46 AM 9/8/2018

# NC DEQ Takes Action

STATE OF NORTH CAROLINA  
COUNTY OF BLADEN  
FILED  
IN THE GENERAL COURT OF JUSTICE  
SUPERIOR COURT DIVISION  
BLADEN COUNTY, N.C.  
17 CVS 580

STATE OF NORTH CAROLINA, et al.,  
MICHAEL S. REGAN, SECRETARY,  
NORTH CAROLINA DEPARTMENT OF  
ENVIRONMENTAL QUALITY,

Plaintiff,

v.

THE CHEMOURS COMPANY FC, LLC,

Defendant.

PARTIAL CONSENT ORDER

NOW THEREFORE, upon the consent of the parties, it is hereby ORDERED,  
ADJUDGED AND DECREED that:

1. Chemours shall continue the measures it has implemented to prevent the discharge of process wastewater containing GenX (HFPD dimer acid) into waters of the State.
2. Chemours shall immediately prevent the discharge of PFBSA compounds referenced in Paragraph 87 of the Complaint in this matter from what Chemours has represented to be the single source of significance in terms of discernible levels of these compounds, and shall continue to prevent the discharge of the same from this source until such time as an NPDES permit with appropriate permit conditions authorizing any such discharge is issued.

State directs Chemours to provide residents with bottled water after GenX found in preliminary well tests

RALEIGH - State officials have directed Chemours to provide bottled water to 11 homeowners near the company's Fayetteville Works facility after the company's preliminary test results showed GenX above state health goals in residential drinking wells.

The state Department of Environmental Quality and Chemours started testing residential wells near the facility after GenX was detected in 13 industrial, non-drinking water wells on the facility's property. Chemours is testing the private wells for GenX. DEQ is testing private wells near the facility also, but in addition to testing for GenX, the state agency is also testing for two other fluorinated compounds, PFOA and PFOS. The state agency is testing for the three fluorinated compounds because they all have established health goals. The state expects its test results in the coming weeks.

To date, Chemours has received preliminary test results for 32 residential wells for people living near the facility. GenX was not detected in 13 residential wells. GenX concentrations were found below the state's provisional health goal of 140 parts per trillion for eight other residential wells. The 11 homeowners with GenX levels above the state's provisional public health goal were supplied with bottled water and health information about GenX. Most of the 11 wells with elevated GenX levels are north of the facility.

The company's test results have not been validated. Out of an abundance of caution, the state directed the company to supply bottled water to residents after receiving word Friday of the first batch of preliminary test results, and then again on Tuesday when Chemours notified the state of another batch of preliminary results.

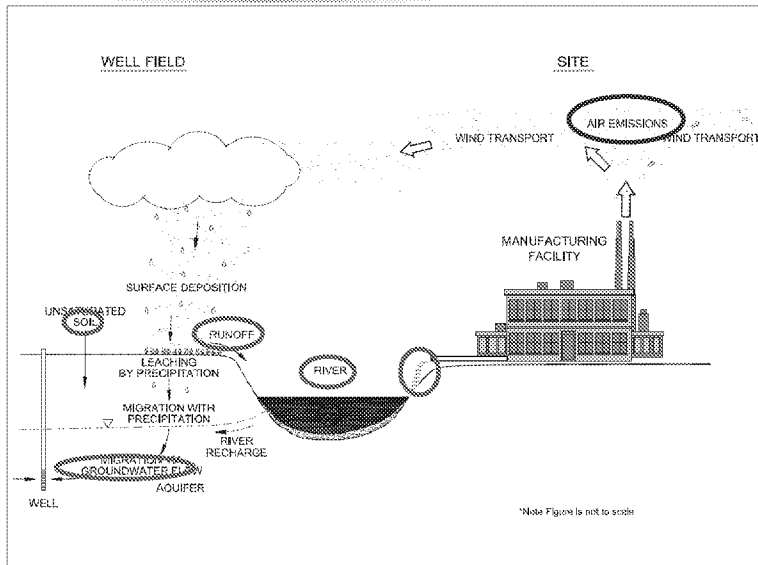
"We want to make sure people with elevated concentrations of GenX in their wells have an immediate alternative water source," said Michael Regan, secretary of the N.C. Department of Environmental Quality. "Making sure people have clean drinking water is our top priority."

This week, DEQ has continued collecting water samples for residential wells nearest the facility. As of Wednesday, DEQ had collected water samples for 31 residential wells in Bladen and Cumberland counties. The state will send samples to Gel Laboratories in Charleston, S.C. for analysis and use the results of testing to determine if people need alternative sources of water and if GenX or the two other fluorinated compounds produced at the facility have moved into the surrounding community. If tests reveal levels of any of the fluorinated compounds above established health goals, the state will direct Chemours to provide affected homes with alternative water and health information on the compounds.

It's important to understand that the state's provisional health goal for GenX represents the concentration of GenX at which no adverse, non-cancer health effects would be anticipated in the most sensitive populations over an entire lifetime of exposure.

Health-related information on GenX, PFOA and PFOS has been posted to DEQ's website at: <https://www.deq.nc.gov/newsroom/press-releases/2016/06/2016-06-20-01>. Residents with questions about the health effects related to GenX, PFOA or PFOS can contact the N.C. Department of Health and Human Services at 919-707-5900.

## Conceptual Model of APFO Emission



Davis et al., 2007 Chemosphere (67) 2011-2019  
 "Transport of ammonium perfluorooctanoate in environmental media near a fluoropolymer manufacturing facility"

## Usual Suspects: Prioritization



- Large peaks
- Found in many samples)
- Contain halogens (Cl, Br, F)
- Negative Mass defect
- Related chemicals

(Kendrick Plots)

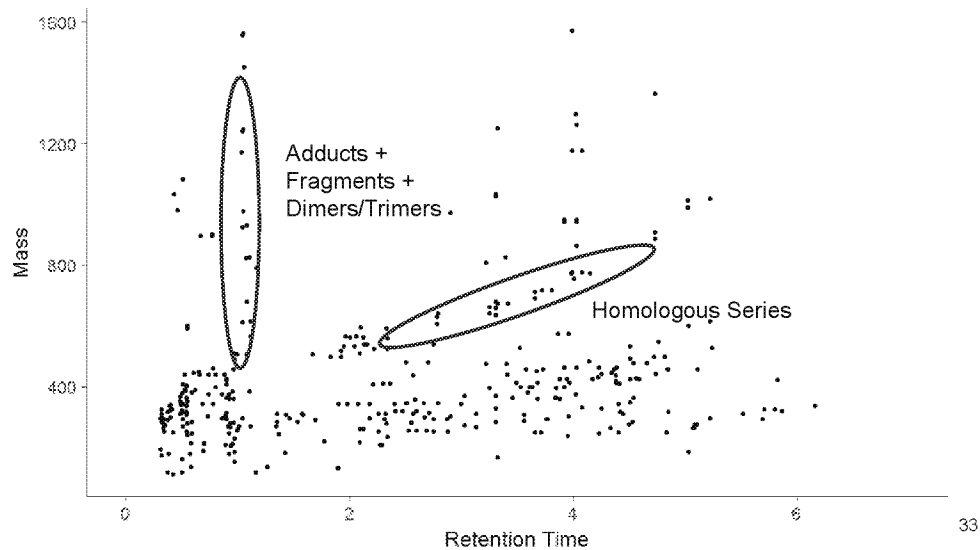


- Diagnostic fragments ( $\text{CF}_3$ -69,  $\text{CF}_3\text{CF}_2$ -119,  $\text{CF}_3\text{O}$ -85,  $\text{CF}_3\text{CF}_2\text{O}$ -135)

U.S. Environmental Protection Agency



## Relationships Reveal Underlying Chemistry



## Homologous Series

